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Charles S. Tomes

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IV. *On the Development of Marsupial and other Tubular Enamels, with Notes upon the Development of Enamel in General.*

By Charles S. Tomes, *M.A.*, *F.R.S.*

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[Plate 16.]

In the year 1849 my father, the late Sir John Tomes, (1) described the structure of Marsupial enamel, showing that the enamel was penetrated, more or less richly, by tubes running into it from the dentine, a thing unusual in other Mammalia. But, in the Marsupials, with the solitary exception of the Wombat, it is universal, although in some, as for example in *Petaurus*, only a few of the tubes enter; and in the Dasyuridæ they are far less abundant, and traverse a smaller thickness of the enamel than in the Macropodidæ.

In the latter, the tubes are exceedingly abundant, most of the dentinal tubes being apparently continued into and through the greater part of the thickness of the enamel; they become finer as they approach the surface, and none of them quite reach it; sometimes an enamel tube is connected with two dentinal tubes, and sometimes (rarely) the converse is the case.

Amongst other Mammals this character is met with occasionally; it may be well seen in the Hyrax (Plate 16, fig. 1); in some Insectivora, notably in the Shrew; in the Jerboa, and in a few other Rodents; whilst in the human subject it may be rarely found, but only in a rudimentary condition (fig. 2).

The facts that the tubes do not reach to the exterior of the enamel, even in *Macropus*; that the penetration is variable, even amongst the Marsupials; that it does not exist at all in the Wombat, and that the character reappears sporadically amongst other Mammals, would seem to render the inference justifiable that striking, and in some respects anomalous, as the character is, it cannot depend upon any very radical difference in the developmental process from that which results in the formation of an ordinary solid enamel.

This inference is borne out by the investigations to be related, which prove to throw much light upon the development of enamels other than those of Marsupials. In those Marsupials in which but few tubes enter the enamel, there is often little or no dilatation at the point of passage, nor is there any marked deviation in direction,

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but in the Macropods there is usually a well-marked dilatation at this point, and there is usually also a sharp bend; there is, in fact, what might almost be described as a clumsy joint; this is noticeable, though to a less extent, in Hyrax.

In *Macropus* there is also a peculiarity in the course of the tubes in their passage through the enamel, at all events in certain regions of the tooth; not only do they pursue gently curved courses, but about one-third of the distance between the surface of the dentine and that of the enamel, they all simultaneously take abrupt bends in more than one plane, which brings them into a course sometimes almost circumferential to the long axis of the tooth; hence, on the same slide they may be seen both in longitudinal and transverse section.

This peculiarity proved to be of great service in unravelling the nature and position of the tubes, as it was possible thus to check the appearances seen in longitudinal section by those apparent in transverse section at closely-adjacent points.

My father (2) had discovered that if a section of a Kangaroo's tooth be ground thin and then submitted upon a slide to the action of an acid, the tubes, or as he supposed, the dentinal fibrils ('Phil. Trans.,' 1856) could be seen hanging out as a fringe from the edge of the dentine, the intervening portions of the enamel having been dissolved. In repeating his experiment I found that the tubes so isolated stain readily with nigrosin (as do also the sheaths of Neumann in the dentine), and that the tubes somewhat readily part from the dentine, remaining upon the slide as a more or less tangled mass, waving about in the fluid; but they cohere strongly together by their enlarged ends where they have touched the dentine. They also resemble the sheaths of Neumann in being resistant to the action alike of acids and of alkalis.

Whether, like the dentinal tubes, they consist of a resistant sheath containing a softer fibril, or whether they wholly consist of the resistant material, has not been shown; but, although the enamel tubes are larger than those of the dentine, it is difficult to get coloured fluids to enter them.

It might have been supposed that it would be a very simple matter to determine the position of these tubes in completed enamel, and to say whether they lay in the interior of the prisms or between them, but as a matter of fact it proved to be very difficult.

For in proportion as the tube system is highly developed, so do the enamel prisms cease to be distinct; indeed, they can hardly be seen at all in those parts of the tooth where the tube system is richly present.

In addition to this, the tubes, like the tubes of dentine, and, indeed, like most enamel prisms, pursue a spiral course, like a corkscrew pulled out until it is nearly straight; hence, even in very thin sections, they appear to wind about with the smallest alteration of focus of the microscope.

It is, however, noteworthy that their spiral excursions do not exceed in amplitude

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the width of an enamel prism, so that the appearances seen in ordinary ground sections would be equally consistent with their winding along in the interior of the prisms, or with their winding round their circumferences in the interstitial substance.

Von Ebner (5) considers that they are certainly between the prisms, an opinion shared by Dr. Paul (10). But, after devoting a great deal of time to the elucidation of this point, I am unable to agree with these observers, and I believe them to lie in the interior of the prisms, a conclusion arrived at alike by the study of completed enamel and of its development.

If a section be ground thin (till it is not much more than 5 μ thick), a troublesome thing to do, owing to the brittleness of enamel, and then very slightly washed with acid, the interstitial substance is acted upon more quickly than the prisms themselves, and the latter come more prominently into view as clear spaces with sharply-defined outlines; but the tubes often cannot be seen at all in sections so treated.

If such a section be stained with nigrosin, the interstitial substance takes the stain, while the prisms themselves do not, but the stain is not very deep, and the substance has a dotted or granular appearance. At the same time, other finer lines, more darkly stained and much more sharply defined (fig. 10), come into view, and these are seen to run in the middle of the prisms, but only for short lengths; by alterations of focus, however, they can be traced further, and it can be seen that their apparent disappearance is due to their following spiral curves, so that only short lengths lie in any one plane.

They are found to wander as far as the interstitial substance, and then to return; moreover, in fortunate places, where the prisms happen to be cut transversely, a dark dot is seen in the centre, or near it, in each mapped-out area (fig. 3). I have, however, only been able to see this last-mentioned appearance in that part of the enamel which lies very near to the dentine, where the tubes are largest.

Although much has been written upon the development of enamel generally, I am not acquainted with any detailed published account of the development of Marsupial enamel, nor, indeed, of any of the forms of tubular enamel.

The material upon which the observations to be recounted have been made has been very kindly placed at my disposal by the Zoological Society, through the kindness of Mr. Beddard, and by Mr. Woodward, of the Royal College of Science; to the latter I am also much indebted for the use of several beautiful serial sections, prepared by him with another end in view, namely, the investigation of the succession of the teeth in Marsupials. My material consisted of embryos of Macropods and of Hyrax, but unfortunately it is almost impossible to procure Marsupial embryos in first-rate histological preservation, as they are apt to have lain in the pouch for a time after their death, and this has added much to the difficulty of getting good sections.

The problem of the method in which the tubular enamels are built up seemed an interesting one, as it was difficult to imagine in what way the epiblastic enamel could

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become penetrated by outgrowths from the mesoblastic dentine, especially when it is remembered that the first thing formed is a thin layer of dentine, in which are contained only the fine terminations of the dentinal tubes, so that the enamel is at the very earliest period cut off from any communication with the dentine pulp, save through these fine canals and their contained fibrils.

To meet this difficulty Dr. PAUL (10) has suggested that tracts of dentine matrix enter and grow out into the enamel, carrying with them the dentinal tubes; but this explanation I am unable to accept for reasons which will become sufficiently clear. But I may say at once that I have come to the conclusion that the tubes of the dentine cannot, in the strictest application of the terms, be said to enter the enamel at all; it is true that the tubes in the enamel are continuous with those of the dentine at the point of junction of the two tissues, but those which lie in the enamel are wholly and entirely a product of the enamel organ, and cannot, therefore, be properly termed "dentinal" tubes.

I have been unable to discover the smallest difference between the enamel organs of Marsupials and those of other Mammals prior to the commencement of calcification.

The first change observed is the formation of a thin layer of dentine, which differs from that ordinarily found in that it has no granular layer, but the dentinal fibrils are continued right out through it to its surface. If it so happens that in cutting the section, or from a little shrinkage in preparation, the enamel cells are parted from the thin first-formed layer of dentine, each enamel cell (hereafter called ameloblast) is found to be furnished with a process, the so-called Tomes' process, described years ago by my father (fig. 6).

At the earliest stage the free extremities of the Tomes' processes are somewhat clubbed, and tend to cohere by their edges; these enlarged ends seem to be concerned in the formation of the thickened joints already alluded to (p. 108), and they account for the cohesion of the tubes at the dentinal surface of the enamel when isolated by the use of an acid. At this period the Tomes' processes seem to become closely applied to, and to perhaps unite with, the terminations of the dentinal fibrils, but they become so quickly imbedded in pits of semi-calcified material that this is difficult to make out with certainty.

As regards their taking stains they always behave precisely as does the plasm of the ameloblasts, of which they are obviously a continuation; and in sections which are very thin, and which happen to hit the axis of a cell and its process, there is no appearance of cell membrane across the end of the ameloblast, but, on the contrary, its interior is continued without break into the process (figs. 6, 8 and 11); the nuclei of the ameloblasts lie near to the further ends of the cells, and are always much more deeply stained than the plasm of the cells.

The Tomes' processes are soft and extensile, so that when the ameloblasts are displaced they may be drawn out into thin threads (figs. 5 and 8), which renders it

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improbable that they have any membranous investment on their sides, although their sharply bordered outlines look as though they had one.

At any but the very earliest stage in development the Tomes' processes are artefact; that is to say, they are short portions of a much longer fibrillar process broken off at a certain uniform distance from the ameloblast, because at this point the impregnation with lime and perhaps other changes have rendered them harder.

The Tomes' process is always, in my preparations, of smaller diameter than its parent cell, in the axis of which it lies, but I am unable to say how far this diminished diameter is due to the drawing out of an extensile substance, or to its shrinkage under the action of reagents; if it could be seen, which it cannot, in a specimen not treated with any reagent, it is probable that it would be nearly or quite of the full diameter of the ameloblast, which about corresponds in size (3·5 μ to 4 μ) to the prism of completed enamel.

The next appearance to which attention is directed, is one that is exceedingly conspicuous in the formation of Marsupial enamel; it is that of a honeycomb, the partitions of which stain strongly with almost all stains, so that the youngest layer of enamel appears to be full of holes or pits (figs. 5 and 8).

The appearances commonly seen are those represented in figs. 5 and 8, but these sections are more or less oblique to the axes of the pits; if the section happens to more closely coincide with their axes, the appearance shown in fig. 11 is seen, where the Tomes' processes are seen to enter and fill up the pits.

To the exact relations of this honeycomb to the ameloblast process I shall have occasion to recur later; for the present, it will suffice to point out a peculiarity in the honeycomb, which is, that it is most conspicuous at a certain stage of enamel formation, that is to say, near to the ameloblasts, but that in the somewhat older enamel it is lost and cannot be seen at all.

This, which is at first sight puzzling, is, nevertheless, easily susceptible of explanation; it is a familiar fact to every student of calcification, that just upon its border line it is very common to meet with a half-calcified material which is very resistant to reagents, and which is very possibly a form of Harting's calcoglobulin (4). Such structures are to be found forming the linings of the dentinal canals as Neumann's sheaths, round the lacunæ of bone, round the Haversian canals of bone, and, as a fenestrated membrane on the surface of developing human enamel when it has been treated with an acid.

But when calcification has proceeded a little further, it loses this power of resistance and dissolves in acids. So that in the case of enamel, we have first the coherent ameloblasts, then a short region in which the Tomes' processes stand isolated from one another, then the honeycomb; and, lastly, in the region of older enamel, isolated fibres occur again in these decalcified sections of tubular enamel, though had an enamel with solid prisms been under observation, nothing at all would have remained here.

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But in the case of a Macropod enamel, long fibres, extending in favourable sections through the whole thickness of the enamel formed and reaching to the dentine, are seen (fig. 9). I use the term fibres for want of a better, but it must not be thought to imply that they have any distinctly fibrous structure; indeed, I can distinguish no structure in them.

The existence of fibres in growing enamel was described by Dr. Andrews (12), in a valuable paper, at the conclusion of which he writes: "I am led to believe that there probably exists in developing enamel, as has already been found in developing bone and dentine, a fibrous substructure on and between which the enamel is deposited. After the enamel is wholly formed, its existence seems to be wholly blotted out in the dense calcification of the tissue." This statement, so far as it goes, I can wholly confirm, so far as solid enamels are concerned. He, however, imagined that they proceeded from the cells of the stratum intermedium, which is certainly not the case.

The fibres are beautifully seen in some of Mr. Leon Williams' photographs, but their full significance has not been recognised owing to their speedy disappearance in the formation of most enamels. In Marsupials, however, owing to the retention of a canal down the axis of the enamel prism, they persist, and can be more effectually studied. When the ameloblast processes have not been torn off at the level of the honeycomb region, they are seen to start out from the axis of each ameloblast, then at a little distance from it to be laterally glued together (fig. 7), and then to become distinct again and to run on to the dentine, getting smaller and more distinct from one another as they go, and sometimes showing very plainly the spiral turns characteristic of the tubes in the enamel, which indeed I believe them to constitute. As confirmatory of the view that the fibres calcify centripetally may be mentioned the fact that within certain limits the older the enamel, *i.e.*, the further from the ameloblasts it lies, the smaller do the fibres, as seen in decalcified sections, appear.

It has been pointed out by Mr. Leon Williams (14) that the long axes of the ameloblasts in many animals by no means correspond with the direction of the prisms of the completed enamel. This is perfectly true, but the direction of the fibres does; hence these often form an angle with the ameloblasts from which they spring.

The ameloblasts stand, roughly speaking, at right angles to the surface of the dentine. That portion of the fine fibre which when broken off is called a Tomes' process, starts in the same direction, but in many places begins to turn off very abruptly about 6 μ from its parent cell, and afterwards undergoes other changes of course (fig. 9). How these changes of direction are brought about there is nothing to show, but I am satisfied that they correspond exactly with the course of the enamel prisms in the finished tissue. And it is possible to trace the fibres into continuity with the plasm contents of the ameloblast (fig. 6), so that we can safely say that each ameloblast sheds off from its open free end, or is converted

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into, plasm which consolidates into a fibre, and that this fibre runs continuously through the whole thickness of the enamel.

To return to the honeycomb structure, it appears that the membranous-looking exteriors of the fibres are fused with the partitions of the honeycomb, as is indicated by that portion of the fibril which intervenes between the ameloblast and the honeycomb shrinking a little so as to leave interspaces, whilst its attached ends do not shrink (fig. 11). This is also well seen where the fibrils have been drawn out, in which case the end of the fibre attached to the honeycomb retains its full diameter, that attached to the ameloblast a considerable part of its diameter, whilst the intervening portion is drawn out into a mere thread (figs. 11 and 8).

Hence it is difficult to resist the conclusion that the septa of the honeycomb are formed by calcification, or by changes preparatory to calcification, in or round the peripheral portions of the fibres, and that this progresses inwards towards their axes, *stopping short, however, in the tubular parts of a Marsupial enamel of reaching their centres, and so leaving a central canal down the axis of each fibre which remains uncalcified.*

In transverse sections of the honeycomb the spaces may sometimes be seen to be occupied by a less deeply stained circular body (fig. 4), though this often does not quite fill the hole. This, however, is probably due, not to such being its normal condition, but to its having shrunk a little where it is still soft, whereas the septa have become rigid, being further advanced in calcification. Oblique sections, though at first sight a little more confusing, lend themselves to the same interpretation.

The ameloblasts, when cut obliquely, are difficult to define, though their nuclei remain conspicuous objects; a little way from their ends the cells of the honeycomb show as ovoid openings (fig. 12), while beyond (above in the figure) the tissue appears more solid.

In the openings of the honeycomb, dark bodies, the fibres cut across, occur in many places, whilst in others they have fallen out; but enough remain to show their identity with the row of oval dark bodies, which, with light interspaces, constitute the older, more solid portion; this is especially well seen in fig. 12, in which it is obvious that the succession in series of dark ovoid bodies are not markings in an individual fibre, but consist of a series of parallel fibres cut across obliquely.

This same appearance is seen in one of Mr. Leon Williams' photographs (fig. 83 of his paper), though he appears to interpret it differently.

It will be noticed that in these oblique sections the partitions of the honeycomb are well stained, as they usually are; but as we progress further into the more formed enamel the corresponding areas become light, and it is the contained fibre alone that is stained. In fact, the septa have almost disappeared under the action of the decalcifying acid; and, still further, in yet more developed enamel, they quite disappear, leaving the fibres free.

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In other words, the most calcified portions dissolve in acid, those less calcified remain and take the stain; while in completed enamel the interstitial parts remain the most susceptible to solution by acids, as is exemplified by the effect of washing a ground section of enamel slightly with weak acid.

Conclusions as to the Development of Marsupial Enamel.

Each ameloblast gives origin to an axial prolongation of its own interior plasm.

The ameloblast is not itself actually calcified, as was formerly supposed by many observers, myself included, but this fibrillar prolongation of its plasm does calcify; hence it seems probable that a single ameloblast gives rise to the whole length of an enamel prism, itself receding as the enamel grows thicker.

In each individual fibre calcification goes on from without inwards, leaving, during the formation of the greater part of the thickness of the enamel, a central tract soft and uncalcified.

Ultimately, however, as the exterior of the enamel is approached, the axial canal becomes smaller and smaller, and finally thins out to nothing, so that a solid prism is the result. This occurs earlier in the process in some Marsupials than in others.

The tracts of calcification belonging to each fibre do not fuse completely with their neighbours, but a small amount of interstitial calcified material is poured out between them.

Nevertheless, it is not at all certain that each fibre has a membranous sheath, although it seems as if their outer were more dense than their inner portions. This theory of the calcification of enamel is quite in accord with the views expressed by Dr. Sims Woodhead (11). He holds that calcification is in a measure a sort of degenerative process, the stages of which are that the active plasm becomes a "formed material," and that in this lime salts, brought and prepared by the still active tissues in its immediate neighbourhood, are deposited, their deposition taking place by physico-chemical processes, which he compares to dialysis.

One difficulty suggests itself in the acceptance of the theory here advocated, which involves the laying down of the enamel prisms as an organic matrix; and this is the chemical composition of enamel.

I have shown elsewhere (15, i.) that the amount of organic matter left in finished enamel, at all events in that of the Elephant, Horse, and Man, is exceedingly small, far less than the three to four per cent. usually given in analyses; and it is hard to understand what becomes of the organic matter which must certainly at one time have been present. I have not succeeded in isolating a sufficient quantity of Marsupial enamel to ascertain with certainty what proportion of organic matter remains in it, but I have met with indications that it is at all events, just as might have been expected, materially more than in the other Mammalian enamels. It is, of course, possible that the fibres, continuous though they are with the plasm of the cells, may

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have already become themselves poor in organic matter. And it is not clear why, unless each fibre has a limiting membrane, the contiguous fibres do not completely fuse with one another; in the most perfect human enamel the prisms are not exceedingly distinct prior to the application of a reagent, but washing with weak acid always renders them so, and it is seen that there is a small amount of interstitial substance which is more quickly acted upon than the prism itself, just as in Marsupial enamel.

I do not propose in this paper to enter into the vexed question of the precise mode in which the lime salts are prepared and deposited, as to do so would extend it to an undue length; whether VON SPEE (7), ANDREWS (12), and WILLIAMS (14) are right in believing that globules of a semi-fluid material rich in lime are formed within the ameloblasts, and shed out from their ends, I am not prepared to say. Several of the photographs and drawings which illustrate this paper, show very plainly the appearance of those oval or spherical forms in the cells upon which their view is founded (figs. 5 and 8).

And a photograph taken by me, many years ago, of the developing enamel of a species of *Halmaturus* seems to lend some support to the idea that there may be some such material, as a vacuolated appearance shown in it is confined to a particular series of sections, and suggests the idea that it has been produced by some sort of coagulation of a semi-fluid material rather than that it is a real histological tissue.

There are, however, some other appearances to which passing attention may be called, as they are shown in the illustrations to this paper.

The ameloblasts vary much in length; of course it has long been known that the enamel organ is more extensive upon the tooth germ than is the area upon which enamel is to be developed, and that the ameloblasts where no functional enamel is going to be formed are short and small.

This variation in the length of the ameloblasts has been alluded to by ALBERTINA CARLSSON (13), when describing the early tooth germs of osseous Fish, but she speaks with reserve as to its significance.

I have noticed, however, that they undergo a great increase in length in Marsupials even after calcification has commenced.

Thus, at first, where but a thin layer has been formed, they are often not more than 30 μ long, whereas in the same or neighbouring sections, where the process is in full swing, they are as much as 45 or 50 μ long; so far as it goes this would point to a single cell forming the whole length of the prism.

Again, the appearance of the nucleus is often peculiar; its usual form is that of an oval, quite filling up the cell at the place it occupies, near to the distal end of the cell.

But, in so large a number of sections as to render it unlikely that it can be due to any accidental cause, it has a square or even a concave end towards the forming enamel (fig. 9), while from the horns of the crescent thus seen in section

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there appear to be processes running down towards the free end of the cell, though of the existence of these delicate fibres I am not very certain.

The plasm of the cell also has a markedly reticulate structure, the threads running principally in its length, but I have not succeeded in connecting them definitely with the developmental process (figs. 5, 6, and 8).

It seems possible, if the nuclei do thus give origin to threads, that their concavity towards the forming enamel may be due to their being more tied at some points than at others, and so assuming this form during their progressive recession as enamel is being formed.

The views advocated in this paper are quite at variance with those expressed many years ago by the late Professor Huxley (3), who believed that the enamel cells or ameloblasts were separated from the forming enamel by a membrane, so that, as calcification took place upon the opposite side of this membrane from that occupied by the cells, they could not take any direct part in the process.

This view has not, more especially of late years, had many adherents, and there is amongst recent writers a tolerably complete consensus of opinion that the enamel cells do take a very primary and active part in the process.

Miss Nunn (6) arguing to a considerable extent upon certain appearances which she had observed in the developing teeth of Plagiostomes, supported Professor Huxley's view, but in some particulars carried it yet further, the following being her conclusions :—

1. The *cuticula dentis* is formed by the metamorphosis, either in whole or in part, of the enamel cells, which have nothing whatever to do directly with the formation of the enamel. In its early stages the cuticula has frequently been considered as " the newly formed layer of enamel " and also as the basement membrane.

2. The basement membrane may be demonstrated upon the surface of the tooth papilla and upon the tooth in all stages of development. It becomes calcified with the other hard tissue of the tooth and cannot be separated by acid.

3. The enamel, like the dentine, owes its origin to the odontoblasts, the processes of which, in an early stage, may be traced quite up to its outer edge.

The nature of the *cuticula dentis*, so long in dispute, seems to have been tolerably conclusively settled by the recent researches of Dr. Paul (9), who considers it to be a thing formed after the completion of the whole thickness of the enamel, and non-existent at any earlier stage in the process. From the shape, size and general appearance of the cell forms which can be traced in it (in describing which he has been to some extent anticipated by Miss Nunn), he concludes that it is formed not by the ameloblasts, but by the cells of the external epithelium of the enamel organ.

If this be the case (and even if his conclusions be not in all respects correct), it would appear that Miss Nunn considers as one and the same thing structures which most observers hold to be two perfectly distinct things, namely, the membrane which can be raised by acid from the enamel of a complete but unworn tooth (Nasmyth's

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membrane or *cuticula dentis*), and that which can be raised by acids from the surface of enamel only during its development.

The latter is not a continuous sheet (15, ii.); at least it is a much perforated sheet, the perforations corresponding in size and position with the ends of the forming enamel prisms, and it is, I believe, entirely artefact, that is to say, it is merely a certain stage in the development of the enamel, of small vertical thickness in most enamels, but, in the form of the "honeycomb" of this paper, of greater thickness in Marsupial enamel.

Nothing which is contained in Miss NUNN's paper appears to me to really militate against this view, and the long fibrillar processes demonstrated in the present paper, which are without question continuous with the ameloblasts, go to prove that there cannot be a membrane separating the ameloblasts from the forming enamel, and also that the ameloblasts do take a most active and direct part in enamel formation.

Miss NUNN's third conclusion, based apparently upon what she had seen in developing Plagiostome teeth, that the enamel owes its origin to the odontoblasts, is startling in being at variance with the almost universally accepted view that the enamel is epiblastic and the dentine mesoblastic in origin.

Nevertheless, from investigations which I commenced only after this paper was in the hands of the Royal Society and which I hope shortly to publish, I believe that, so far as the so-called enamel of Plagiostomes is concerned, she was nearly right, and had hit upon a significant discovery, in which she was partly anticipated by HERTWIG, but which appears to have been overlooked by most subsequent writers.

But so far from this observation being applicable as an argument as to the manner in which enamel generally is developed, I consider that it is to be interpreted quite differently.

The existence of a strongly differentiated layer upon the mesoblastic dentine papilla of Plagiostomes, which HERTWIG and she have quite rightly, though not in much detail, described, I can confirm, and it does certainly participate in the formation of the so-called enamel of these creatures.

But I think that this goes to show that their so-called enamel is not fully homologous with that of higher vertebrates, for neither this layer itself nor anything corresponding to it has any existence in the Mammalian tooth germ.

And, indeed, the suspicion that the enamel of Plagiostomes was not fully comparable to that of Mammals seems to have occurred to writers who have only investigated it in its completed condition; thus the late Sir RICHARD OWEN gave to it a special name, that of *Ganoin*, and more recently JAECKEL (16) speaks of it as a very low form of enamel, and proposes for it the name *Placoinschmelz*.

But I have only very lately adopted the view that the enamel of Plagiostomes is not fully homologous with that of Mammals; and, indeed, in this paper, as originally presented to the Royal Society, I had drawn comparisons between the tubular

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enamels of Marsupials and of Plagiostomes which I no longer consider to hold good in their entirety.

The theory of enamel formation here advanced, whilst reconcilable with the figures given by Röse (8), is not quite in accord with the views recently advocated by Leon Williams (14), who is of opinion that the enamel prism (or rod as he generally styles it) is the thing first formed, and that the prisms are at a later stage of the process fused together by an at first semi-fluid material, which runs in between them; whereas the illustrations which accompany this paper would indicate that the interstitial substance and the peripheries of the prisms are the first things to be formed.

And whilst I admire the beauty of his specimens and of the photographs which illustrate his paper, I cannot help thinking that if he had had Marsupial enamel under observation, he would have drawn somewhat different conclusions as to the development of enamel generally.*

The view that enamel formation essentially consists in the centripetal calcification of preformed fibres, is applicable to all enamels, and serves to render intelligible many facts that were otherwise difficult to understand. Tomes' processes and the existence of a thin sheet of young enamel, which was perforated, have long been recognised

* Mr. Leon Williams (14, i.) writes: "If we look closely at the last layer of developing enamel shown at *d*, we shall see that it contains a row of globular bodies of very regular and uniform size. These are manifestly identical with those seen along the edges of the enamel in the acid-treated specimens shown in figs. 33 and 35 of this paper. They are quite distinct from the masses of calcoglobulin, such as are seen at C, in fig. 38. In fact, they are often, I may say generally, obscured by the melting together of the large irregular masses. The enamel rods are built up by the successive, rhythmical orderly deposit of these bodies of uniform size, which I shall henceforth denominate the enamel globules. The larger, more transparent, and irregularly sized bodies of calcoglobulin melt or flow together to form the interprismatic substance"(p. 36).

"There are two distinct products of the enamel-forming organ. One of these products, from which the enamel rods are built up, is formed by the ameloblasts, and is probably a direct nuclear formation. In the enamel cells it takes the form of globular bodies containing granules, sometimes arranged with more or less order, so as to resemble the nucleus of the cell. In the formed enamel rod these globular bodies are more or less compressed into disk-like shapes, and are sometimes nearly or quite melted into one another. Simultaneously or alternately with the deposit of the globular bodies a translucent substance like albumen in appearance is seen passing out of the ameloblasts. This substance is probably taken from the blood by the secreting cells of the *stratum intermedium*, and evidently contains the mineral matter of which the completed enamel consists. As the globular bodies pass from the ameloblasts they are seen to be connected with plasm strings, which strings can often be plainly seen in the body of the ameloblasts. The globular bodies are also often seen connected literally by strings or projecting processes. Around the skeleton thus formed, which constitutes the real structure of enamel, the albumen-like substance flows, supplying the cement substance and probably the mineral matter, for the calcification of the whole" (p. 78).

But Mr. Leon Williams in his earlier writings upon the subject (14, ii.) came, in my opinion, very close to the true interpretation of the development of enamel, for he there wrote that in teased-out enamel cells a fibre runs out from the end of the cell, and that as the formation of enamel progresses the cells recede, leaving within the formed enamel what appears to be a fibre of living matter in the centre of the enamel prism.

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both in human and other Mammalian enamels, and, indeed, traces of a honeycomb structure may often be seen. So also have the soft fibres been described by ANDREWS and others, but the full significance of these several structures has not been recognised, owing to their being so speedily obscured by the complete solid calcification of the enamel prism.

Once grant that a manner of calcification, resulting in the formation of tubes, is the normal and indeed the universal procedure in the first instance, and that the differences are solely in degree; that is to say, that the tube system is retained for the greater part of the thickness of the enamel, but is abandoned in favour of a solid calcification in the outermost part of the enamel, in Marsupials, and that in the rest of Mammalia the tube condition is very transient, the fibres speedily becoming solid prisms, then the difficulty of accounting for the occasional occurrence of tubes in human enamel (fig. 2), and for the reappearance of the tubes in certain Rodents, Insectivora, and in Hyrax (fig. 1), disappears, and it is not necessary, as heretofore, to seek a teleological interpretation for a structure that we cannot see to be in any way advantageous: it becomes simply the retention of a stage of development through which all alike pass, and which some retain and others do not.

The calcifying fibres seem to present some parallel with ordinary osteogenetic fibres, and perhaps, in respect of their imperfect calcification, with SHARPEY's fibres, but they stand alone in being the product of epithelial cells, and also perhaps in being distinctly the extensions of individual cell contents.

Tubular enamels are quite common amongst Fish, but until their development has been worked out, it is unsafe to draw close comparisons with them; for at present it appears as though in them, or at all events in Plagiostome fish, a tissue which, when complete, has a good deal of resemblance to a Marsupial tubular enamel, has been formed by a developmental process by no means identical.

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Lettering Applicable to all the Figures.

a. Ameloblasts.
b. Cells of *stratum intermedium*.
c. Youngest portion of enamel on honeycomb region.
d. Dentine.
d.p. Dentinal pulp.
e. Enamel.
f. Fibrillar processes of the ameloblasts.
g. Tubes in the enamel.
h. Boundaries of the enamel prisms.
i. Enamel prisms tolerably advanced in calcification.
k. Globular bodies of von Spee and Andrews.
l. Tomes' processes.
s. Stellate reticulum of the enamel organ.

Description of Plate 16.

Fig. 1. Ground sagittal section of tooth of Hyrax; a slight bend and dilatation exists where the tubes pass from the dentine into the enamel. × 80. From a photograph.

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Fig. 3. Ground section of the enamel of *Dasyurus*, near to the dentine, and parallel with its surface, so as to cut the enamel prisms transversely; slightly washed with acid. × 1000. Drawing.

Fig. 4. Section of young enamel of *Halmaturus* transverse to the cells of the honeycomb, decalcified and stained. The slightly shrunken fibrils are seen occupying the spaces. × 700. Drawing.

Fig. 5. Developing enamel of *Macropus*. The section is not quite parallel with the long axes of the ameloblasts, hence the cells of the honeycombed layer are opened somewhat obliquely. The ameloblasts are slightly pulled away from the forming enamel, so that their processes are drawn out and thinned down. × 700. From a photograph.

Fig. 6. Ameloblasts of *Macropus*, showing Tomes' processes and their continuations into long fibrils. At one side (the left) the section does not pass through the axis of the cell and its process, and here there is an appearance of a membrane across the end of the cell, which is absent at the other side. × 700. From a drawing.

Fig. 7. A general view of the developing tooth of *Halmaturus*. To the right, near the top, is the stellate reticulum of the enamel organ, then the *stratum intermedium*, the ameloblasts, Tomes' processes, and the longer fibrils into which they are continued. Below this comes a layer of dentine, enclosing the dentinal pulp. × 200. From a photograph.

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Fig. 10. A ground section of the enamel of *Macropus*, which has been washed with acid and stained with nigrosin. The tubes are seen as short, curved, sharply defined lines, whilst the interstices of the prisms are less deeply stained. × 500. From a drawing.

Fig. 11. A somewhat oblique section of ameloblasts, their processes, and the pits of the honeycomb. In this section there appears to be some differentiation of the axial portions of the fibrils, as ill-defined dark areas appear in their centres. × 800.

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Fig. 12. An oblique section through the ameloblasts, which are at the bottom of the figure; the honeycomb region; and, at the top of the figure, somewhat more advanced enamel. In this latter region many rows of parallel prisms or fibrils are cut obliquely, so that a pattern which does not belong to a single fibril or prism is produced. The interstices between them are here clear and unstained. × 650. From a photograph.

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Fig. 1. x 80

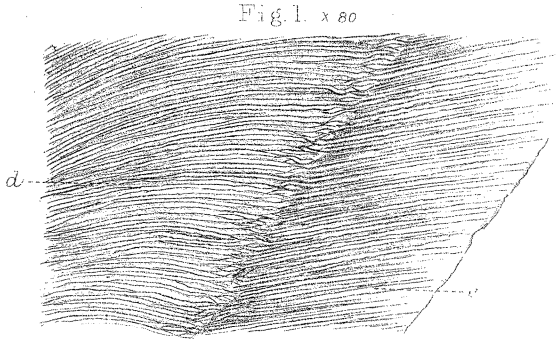


Fig. 2. x 200

Fig. 4.

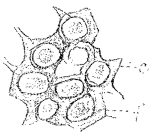


Fig. 3. x 1000

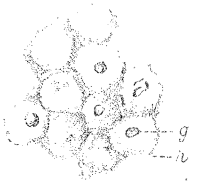


Fig. 5. x 700

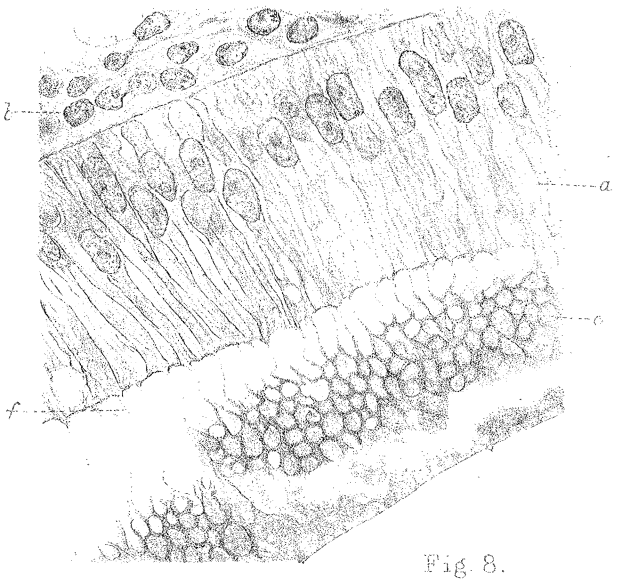


Fig. 7. x 200

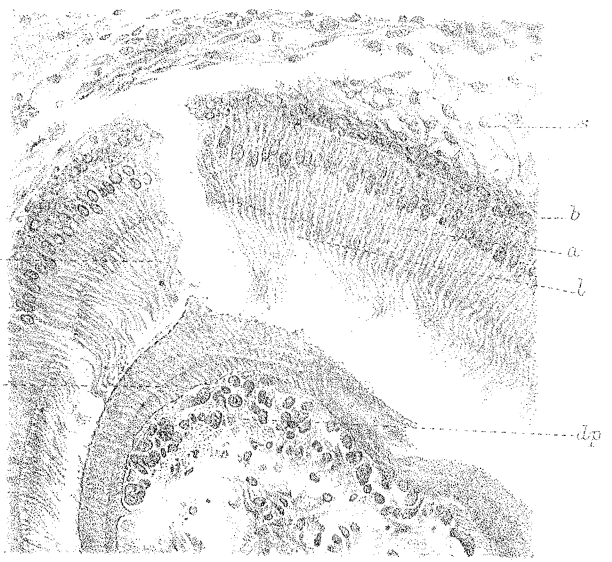


Fig. 8.

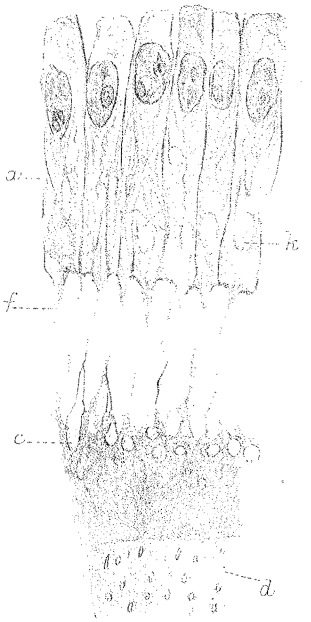


Fig. 6.

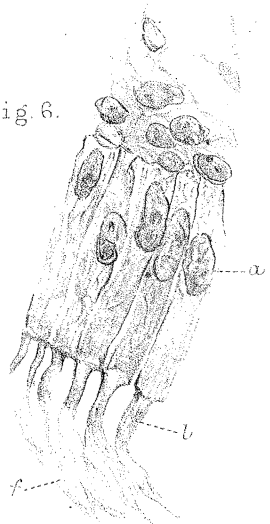


Fig. 9. x 250

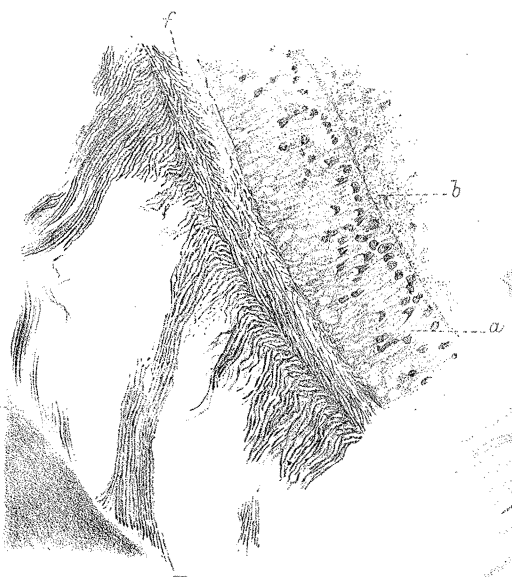
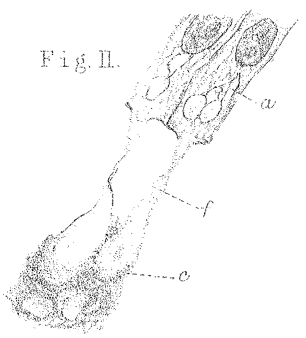


Fig. 11.



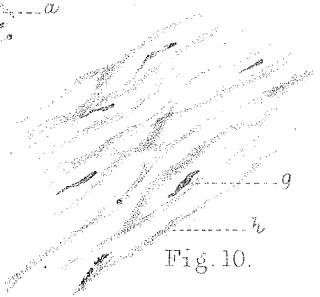


Fig. 10.

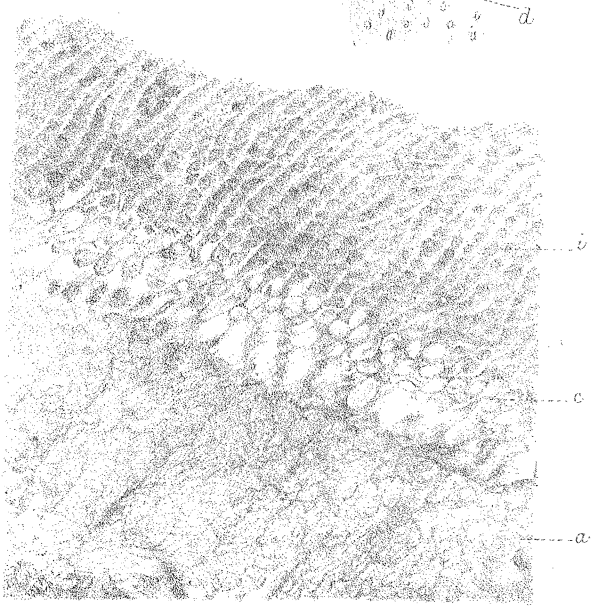


Fig. 12. x 650

West, Newman lith.

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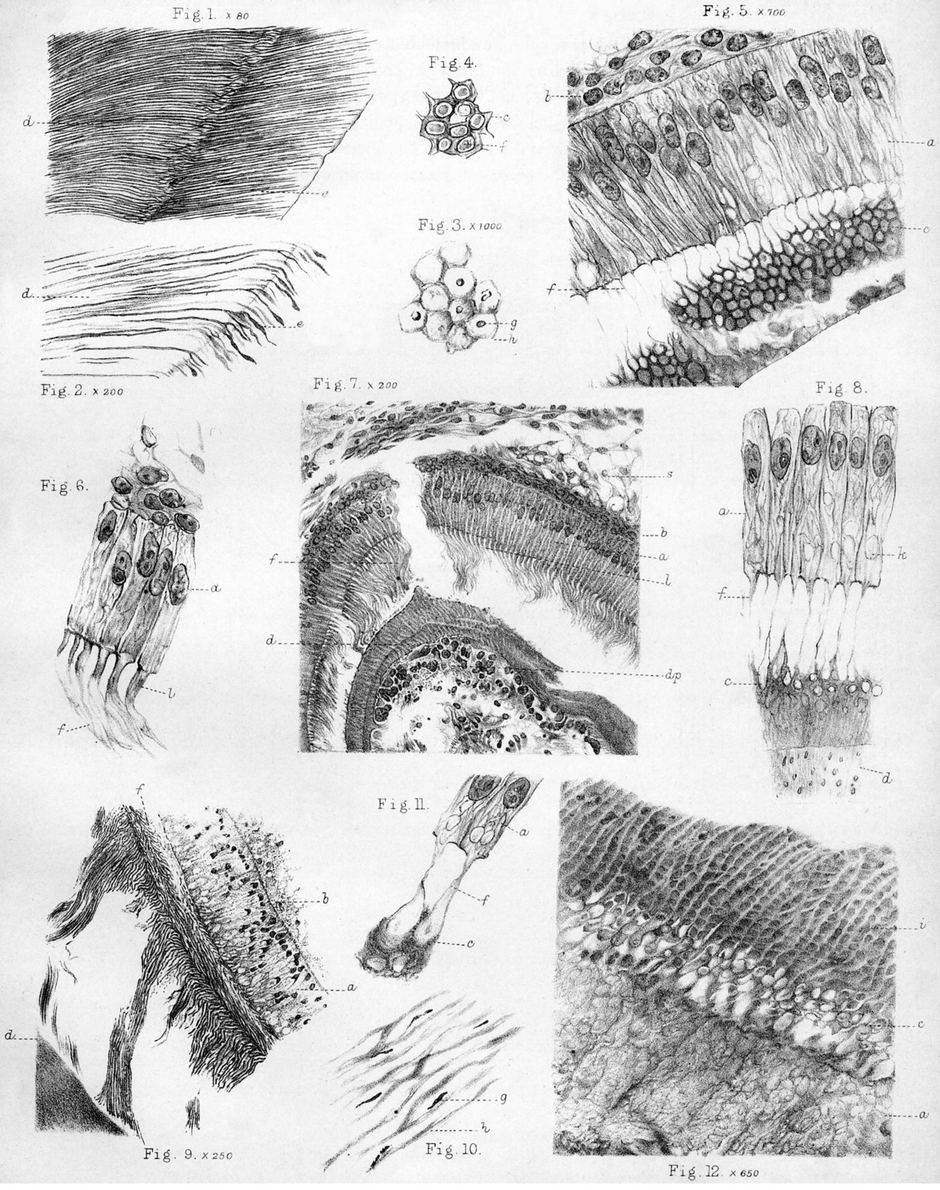

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